UNIVERSITI PUTRA MALAYSIA

CHARACTERIZATION OF GRANULOVIRUS AND NUCLEOPOLYHEDROVIRUS ISOLATED FROM *SPODOPTERA LITURA*

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By

LAU WEI HONG

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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Specially to my husband, my mother, my brothers and sister

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

CHARACTERIZATION OF GRANULOVIRUS AND NUCLEOPOLYHEDROVIRUS ISOLATED FROM *SPODOPTERA LITURA*

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March 2002

Chairman: **Professor Norani Abdul Samad, Ph.D.**

Faculty: **Science and Environmental Studies**

Two baculoviruses were isolated and identified from *Spodoptera litura*; S. litura nucleopolyhedrovirus (SpltNPV) and S. litura granulovirus (SpltGV). The polyhedra of SpltNPV were about 0.9-1.83 μm in diameter containing multiple virions measuring about 100-280 nm wide and 320-410 nm long. The SpltNPV virions contained nucleocapsids (47-60 nm wide and 300-350 nm long) within an envelope, and the size of capsids measured about 58-60 nm wide and 300-330 nm long.

The capsules of SpltGV were about 0.2-0.3 μm wide and 0.45-0.55 μm long containing single virion (60-73 nm wide and 245-267 nm long). The SpltGV nucleocapsids measured approximately 54-60 nm wide and 287-410 nm long, and found singly enclosed within an envelope. The SpltGV capsids measured about 36-58 nm wide and 175-277 nm long.

The restriction endonuclease analyses (REN) revealed that these two baculoviruses did not show any identical restriction pattern. The DNA size of the SpltNPV and the

SpltGV was estimated to be 132 kb and 124 kb, respectively. The nucleotide sequence analysis of the polyhedrin gene of SpltNPV had 98% sequence identity to the known SpltNPV (accession number: AF037262); while the granulin gene of SpltGV had 81% sequence identity to the granulin gene of Xestia c-nigrum granulovirus (accession number: U70069). Based on the sequence analysis, the SpltNPV and the SpltGV are placed as a taxon of Group II NPV and Group GV, respectively.

Both viruses exhibited general symptoms of polyhedrosis and granulosis. The SpltNPV-infected larvae showed pinkish yellow at the dorsal and lateral sides, while the SpltGV-infected larvae exhibited whitish ventral. The SpltNPV caused a reduction in the larval size while the SpltGV-infected larvae increased in size with bloated integument when lower viral dosages were given. Both viruses infected fat bodies, Malphigian tubules, tracheal matrices, hypodermis, muscles and midguts. The SpltNPV replicated in the nucleus and spread the disease to susceptible tissues within 24-h postinoculation (p.i). The SpltGV was found replicating in both nucleus and cytoplasm, and the disease spread gradually after 48-h p.i. The LD_{50} of both viruses in neonate larvae of *S. litura* were $9.04x10^2$ polyhedra for SpltNPV and $1.26x10^4$ capsules for SpltGV. The LT_{50} of both viruses were similar when neonate larvae were fed with similar ranges of viral dosages. The SpltNPV showed a higher virulence in *S. litura* larvae than the SpltGV. The characterization of these baculoviruses is of particular interest in view of its possible use in biological or integrated control.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENCIRIAN GRANULOVIRUS AND NUCLEOPOLYHEDROVIRUS DARI *SPODOPTERA LITURA*

Oleh

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Dua jenis bakulovirus telah dipencilkan and dicirikan dari *Spodoptera litura*, iaitu S. litura nukleopolihedrovirus (SpltNPV) dan S. litura granulovirus (SpltGV). Saiz polibedra SpltNPV adalab lebih kurang 0.9-1.83 μm diameter dan mengandungi virion berganda yang berukuran 100-280 nm lebar and 320-410 nm panjang. Virion SpltNPV mengandungi nukleokapsid (47-60 nm lebar dan 300-350 nm panjang) dalam satu sampul dan kapsid berukuran 58-60 nm lebar dan 300-330 nm panjang.

Kapsul SpltGV adalah lebih kurang 0.2-0.3 μm lebar dan 0.45-0.55 μm panjang dan mengandungi satu virion (60-73 nm lebar dan 245-267 nm panjang). Saiz nukleokapsid SpltGV lebih kurang 54-60 nm lebar dan 287-410 nm panjang dan satu nukleokapsid terdapat terkurung dalam satu sampul. Kapsid SpltGV adalah berukuran 36-58 nm lebar dan 175-277 nm panjang.

Analysis Pembatasan Endonuklease (REN) menunjukkan kedua-dua bakulovirus tersebut tidak mempunyai corak pembatasan yang sama. Saiz DNA SpltNPV dan SpltGV telah dianggarkan sebesar 132 kb dan 124 kb, masing-masing. Analysis jujukan nukleotida menunjukkan gen polihedrin SpltNPV mempunyai 98% homologi dengan SpltNPV yang dikenali (nombor asesi: AF037262), manakala gen granulin SpltGV mempunyai 81% jujukan sama dengan gen granulin XcGV (nombor asesi: U70069). Berdasarkan analysis jujukan tersebut, SpltNPV dan SpltGV masing-masing diletakkan sebagai satu takson dalam Kumpulan NPV II dan Kumpulan GV.

Kedua-dua virus menghasilkan simtom penyakit polihedrosis dan granulosis yang umum. Larva yang dijangkiti oleh SpltNPV menunjukkan warna kuning kemerah-mudaan pada sisi-sisi tepi dan belakang, manakala larva yang dijangkit oleh SpltGV menunjukkan warna putih pada sisi ventral. SpltNPV menyebabkan pengurangan saiz larva, manakala larva yang dijangkiti oleh SpltGV bertambah saiz badan dengan integumen yang mengembang apabila sukatan virus yang rendah diberikan. Kedua-dua virus menjangkiti tisu lemak, tubul Malfigian, matriks trakea, hipodermis, otot dan usus tengah. SpltNPV membiak dalam nukleus dan penyakit merebak ke tisu-tisu yang mudah dijangkiti dalam masa 24 jam selepas jangkitan (p.i.). SpltGV didapati membiak dalam kedua-dua nukleus dan sitoplasma dan penyakit merebak secara perlahan-lahan selepas 48 jam jangkitan (p.i.). LD_{50} untuk kedua-dua virus dalam larva *S. litura* yang baru lahir adalah $9.04x10^2$ polihedra untuk SpltNPV dan $1.26x10^4$ kapsul untuk SpltGV. LT_{50} adalah sama untuk kedua-dua virus bila larva yang baru lahir diberi julat sukatan virus yang serupa. SpltNPV menunjukkan kevirulenan yang lebih tinggi daripada SpltGV terhadap larva *S. litura*. Pencirian

kedua-dua baculovirus tersebut adalah diminati khas disebabkan penggunaannya dalam pengawalan biologi atau bersepadu.

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I certify that an Examination Committee met on 11th March 2002 to conduct the final examination of Lau Wei Hong on her Doctor of Philosophy thesis entitled "Characterization of Granulovirus and Nucleopolyhedrovirus Isolated from *Spodoptera litura*" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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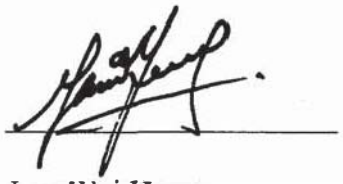
Date: 09 MAY 2002

DECLARATION

I hereby declare that the thesis is based on my original work except for the quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



Lau Wei Hong

Date: 20/3/2002

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

A260	absorption at 260 nm
AcfeMNPV	Actebia fennica MNPV
AcMNPV	Autographa californica MNPV
AcMNPV	Autographa californica MNPV
AgMNPV	Anticarsia gemmatalis MNPV
AgNPV	Anticarsia gemmatalis NPV
AgurSNPV	Aglais urticae SNPV
AjGV	Achaea janata GV
AnfaMNPV	Anagrapha falcifera MNPV
AnpeNPV	Antheraea pernyi NPV
ArceMNPV	Archips cerasivoranus MNPV
ArceNPV	Archips cerasivoranus NPV
ArveGV	Argyrotaenia velutinana GV
BmMNPV	Bombyx mori MNPV
BmNPV	Bombyx mori NPV
bp	base pairs
BusuNPV	Buzura suppressaria NPV
BusuSNPV	Buzura suppressaria SNPV
CfNPV	Choristoneura fumiferana NPV
ChfuGV	Choristoneura fumiferana GV
ChmuGV	Choristoneura murinana GV
ChroMNPV	Choristoneura rosaceana MNPV
CpGV	Cydia pomonella GV
CrleGV	Cryptophlebia leucotreta GV
DiheSNPV	Diprion hercyniae SNPV
DNA	deoxyribonucleic acid
dNTP	deoxynucleoside triphosphate
EcobSNPV	Ectropis obliqua SNPV
EDTA	ethylenediamine tetraacetic acid
EppoMNPV	Epiphyas postvittana MNPV
EppoNPV	Epiphyas postvittana NPV
ErtiSNPV	Erannis tiliaria NPV
EsacGV	Estigmene acrea GV
EuocGV	Euxoa ochrogaster GV
ExapGV	Exartema appendiceum GV
GlbiGV	Glena bisula GV
GV	Granulovirus
h	hour
HabrGV	Harrisina brillians GV
HearNPV	Helicoverpa armisgera NPV
HycuGV	Hyphantria cunea GV
HycuNPV	Hyphantria cunea NPV
HzNPV	Helicoverpa zea NPV
HzSNPV	Helicoverpa zea SNPV
HzSNPV	Helicoverpa zea SNPV

JucoGV	Junonia coenia GV
kb	kilobase pairs
LD_{50}	lethal dose 50
LdNPV	Lymantria dispar NPV
LeseNPV	Leucania seperata NPV
LT_{50}	lethal time 50
LymoNPV	Lymantria monacha NPV
M	micromolar
MaamMNPV	Malacosoma americanum MNPV
MacoNPV	Malacosoma constrictum NPV
MadiMNPV	Malacosoma disstria MNPV
MARDI	Malaysian Agricultural Research and Development Institute
MbMNPV	Mamestra brassicae MNPV
MbNPV	Mamestra brassicae NPV
MepeGV	Melanchra persicariae GV
mm	minute
MNPV	multiple nucleocapsid polyhedrosis virus
NanaGV	Natada nararia GV
Nese SNPV	Neodiprion sertifer SNPV
Nesw SNPV	Neodiprion swainei NPV
Nevi SNPV	Neodiprion virginiana SNPV
nm	nanometer
NPV	Nucleopolyhedrovirus
nt	nucleotide
OpMNPV	Orgyia pseudosugata MNPV
OpNPV	Orgyia pseudosugata NPV
OranNPV	Orgyia anartoides NPV
Orat SNPV	Orgyia antiqua SNPV
Orle SNPV	Orgyia leucostigma NPV
p.i.	post-inoculation
PadoNPV	Panaxia dominula NPV
PaflMNPV	Panolis flammea NPV
PalaMNPV	Pandemis lamprosana NPV
PbGV	Pieris brassicae GV\
PCR	polymerase chain reaction
PenuNPV	Perina nuda NPV
PhopGV	Phthorimaea operculella GV
PIB	Polyhedral Inclusion Body
PiraGV	Pieris rapae GV
PlorNPV	Plusia orichalcoa NPV
PlscGV	Plathypena scabra GV
Probit	probability unit
PsunGV	Pseudaletia unipuncta GV
PyanGV	Pygaera anastomosis GV
RoMNPV	Rachiplusia ou MNPV
s	second
SacaGV	Sabulodes caberata GV

SDS	sodium dodecyl sulfate
SeMNPV	Spodoptera exigua MNPV
SeNPV	Spodoptera exigua NPV
SfMNPV	Spodoptera frugiperda MNPV
SfNPV	Spodoptera frugiperda NPV
SNPV	single nucleocapsid polyhedrosis virus
SpliMNPV	Spodoptera littoralis MNPV
SpltNPV	Spodoptera litura NPV
TAE	Tris-acetate-EDTA buffer
Taq	Thermos aquaticus
ThliSNPV	Thymelicus lineola SNPV
ThorSNPV	Thysanoplusia orchalcea SNPV
TipaNPV	Tipula paludosa NPV
TnGV	Trichoplusia ni GV
TnSNPV	Trichoplusia ni SNPV
TrvmSNPV	Trichiocampus viminalis SNPV
V	volt
v/v	volume per volume
w/v	weight per volume
WisiNPV	Wiseana signata NPV
WisiSNPV	Wiseana signata SNPV
XcGV	Xestia c-nigrum GV

CHAPTER 1

INTRODUCTION

An insect is considered a pest when its presence causes an economically important loss. Various types of chemicals have been used for controlling pests. They are fast in action and can be used for a broad range of pests. The excessive use of chemical insecticides, however, can result in building up of pest resistance, side effects on beneficial and non-targeted insects, and pollution to the environment that indirectly harm public health. Chemical control has worsened the pest problem.

Integrated pest management (IPM) promotes an alternative to chemical pest control which includes the use of pest-resistant plants, cultural methods, and biological control, and recommends the application of combined methods to minimize the chance of the target insects adapting to any single tactic (Zechendorf, 1995). Biological control utilizes natural living organisms to control a particular pest. These natural enemies can be a predator or parasite (macrobial control), or pathogen (microbial control) (Burges and Hussey, 1971). They normally occur under the conditions of pest outbreaks and are important factors in reducing the density of pest under natural population (Weiser, 1977). These biological control agents neither accumulate in the food chain nor harm the environment, but only make contact with particular targets. According to Debach and Rosen (1991), only 15% of these organisms have been discovered and identified.

The use of microbial control in insect pest is not a new concept. Microbial insecticides mainly refer to bacteria, fungi, viruses, nematodes, protozoa and rickettsiae. *Bacillus thuringiensis* toxin (Bts) is the most successful bioinsecticide and commercially available. However, resistance of pest against the Bt has been reported (Zechendorf, 1995). Fungi, protozoa and nematodes are slow in action and only effective under favorable conditions. Rickettsiae have low specificity to its host and are also pathogenic to warm-blooded animals (Burges and Hussey, 1971). Therefore, viruses are promising biological control agents since they attack the target insects and reprogram the host cells for virus production. Death is the final stage for insect with viral disease.

Baculoviruses are the most commonly and widely studied double-stranded DNA viruses that infect insects. They are divided into *Nucleopolyhedrovirus* (NPV) and *Granulovirus* (GV) based on their morphology. The baculoviruses have been found in over 600 species of arthropods (Blissard and Rohrmann, 1990) and can be as effective as chemical pesticides in controlling specific pests. They are environmentally attractive because they are highly host-specific and, in general, only infect insect species within a limited host range. They have no impact on plants, mammals, fishes, birds or even non-target insects and do not accumulate in food chains. The occluded viruses are very stable and transmitted horizontally among their host. They are ingested by the susceptible larvae, replicate in the host and finally the host dies releasing large quantities of the occlusion bodies into the environment that maximize the chance of other insects to come in contact with the virus and in turn become infected. Vertical transmission, however, occurs through contamination of

female ovipositor during egg laying. The newly hatched larvae will be infected after consuming the virus from the eggshell.

The development of baculoviruses as an ideal IPM candidate is very promising in the near future. Since people are very concerned on the impact of using chemical control, many baculoviruses have been discovered and studied. Miller and Dawes (1978) reported that HzNPV and OpNPV were registered as pesticides by the U.S. Environmental Protection Agency.

In Malaysia, NPV and GV have been found naturally in the diseased larvae of *Spodoptera litura*. A pathogenicity test of SpltNPV to *S. litura* larvae was carried out by Sajap *et al.* (2000) who reported that the larval mortality was dependent on the viral doses. The properties of NPV and GV found in *S. litura* larvae have not been fully characterized yet. The fundamental studies of these viruses are important to commercial production. Furthermore, these studies are crucial in managing and manipulating the viruses. Since insect virus classification is based on the intrinsic properties of the virus, this thesis concentrates on the basic characterization of both viruses in order to develop a better biopesticide that is environmentally friendly and highly effective for controlling *S. litura*. Therefore, the objectives to this study are to:

1) Isolate and characterise GV and NPV from *Spodoptera litura*.
2) Analyse the DNA of GV and NPV from *S. litura*.
3) Study the pathology of GV and NPV in *S. litura*.